Effects of inhaled fine dust on lung tissue changes and antibody response induced by spores of opportunistic fungi in goats

Charles W. Purdy, DVM, PhD; Robert C. Layton, PhD; David C. Straus, PhD; J. R. Ayers, DVM, PhD

Objective—To investigate the effects of sterile fine dust aerosol inhalation on antibody responses and lung tissue changes induced by *Mucor ramosissimus* or *Trichoderma viride* spores following intratracheal inoculation in goats.

Animals—36 weanling Boer-Spanish goats.

Procedures—6 goats were allocated to each of 2 *M ramosissimus*–inoculated groups, 2 *T viride*–inoculated groups, and 2 control (tent or pen) groups. One of each pair of spore-treated groups and the tent control group were exposed 7 times to sterilized fine feedyard dust (mean ± SD particle diameter, < 7.72 ± 0.69 µm) for 4 hours in a specially constructed tent. Goats in the 4 fungal treatment groups were inoculated intratracheally 5 times with a fungal spore preparation (30 mL), whereas tent control goats were intratracheally inoculated with physiologic saline (0.9% NaCl) solution (30 mL). Pen control goats were not inoculated or exposed to dust. Goats received an IV challenge with equine RBCs to assess antibody responses to foreign antigens. Postmortem examinations were performed at study completion (day 68) to evaluate lung tissue lesions.

Results—5 of 7 deaths occurred between days 18 and 45 and were attributed to fine dust exposures prior to fungal treatments. Fine dust inhalation induced similar lung lesions and precipitating antibodies among spore-treated goats. Following spore inoculations, dust-exposed goats had significantly more spores per gram of consolidated lung tissue than did their nonexposed counterparts.

Conclusions and Clinical Relevance—Fine dust inhalation appeared to decrease the ability of goats to successfully clear fungal spores from the lungs following intratracheal inoculation. (*Am J Vet Res* 2008;69:501–511)

Aerosol particulates and their relation to human health[1–3] and perhaps health of other animals[4] are of interest to the Environmental Protection Agency. Regulations are in place for aerosol particles ≤ 10 µm in diameter,[2] and recently, particle sizes of ≤ 2.5 µm in diameter[2,3] have been deemed particularly toxic to humans on the basis of epidemiologic evidence.[5–7]

In a previous study[8] by our group, *Mucor ramosissimus* and *Trichoderma viride* administered via the trachea were found to be virulent in nonimmunocompromised goats that had been exposed to aerosolized sterilized coarse feedyard dust. On the basis of this finding, it was hypothesized that inhalation of aerosolized fine dust prior to fungal exposure would increase the virulence of *M ramosissimus* and *T viride* and enhance their pathogenic processes in goats, compared with findings in fungus-treated goats that were not previously exposed to dust. There is controversy over why the smaller particles are more toxic. The proposed theories regarding toxicity focus on the size of the particles[9] and their chemical composition.[10] The most obvious difference is that smaller particles are respirable (≤ 5 µm in diameter) and can reach the alveoli of the lungs, whereas larger particles are nonrespirable (10 µm in diameter) and probably do not pass beyond the nasal passages or sinuses. However, both aerosolized particle sizes are capable of inducing an inflammatory response[11] when the dust originates from manure and contacts the mucous membranes of a host.[12–14] The inflammatory response is induced by endotoxin,[15] a lipopolysaccharide that originates from the outer cell membrane of gram-negative bacteria, which are plentiful in manure.[16]

Among humans, the reported number of fungal infections and associated mortality rates are increasing. Each year, the proportion of immunoincompetent people within the population appears to increase, largely as a result of the spread of HIV infections and administrations of modern immunosuppressive drugs for transplantation of organs[17] and the treatments of other diseases.[18] However, although rare, the frequency of fungal infections in apparently immunocompetent humans is

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From the USDA, Agricultural Research Service, Conservation and Production Research Laboratory, PO Drawer 10, Bushland, TX 79012 (Purdy); the Department of Microbiology and Immunology, School of Biomedical Sciences, Texas Tech University Health Sciences Center, Lubbock, TX 79430 (Layton, Straus); and Veterinary Diagnostic Laboratory, Texas A&M University, Amarillo, TX 79106 (Ayers). Dr. Ayers' present address is Veterinary Diagnostic Center, Department of Veterinary Biomedical Sciences, University of Nebraska, Lincoln, NE 68583.
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Address correspondence to Dr. Purdy.

also increasing.[19-23] Immunocompetent mice have been used to grade the relative virulence of *Candida* spp on the basis of numbers of deaths and kidney infections following inoculation with the yeasts.[24] The purpose of the study reported here was to investigate the effects of sterile fine dust aerosol inhalation on antibody responses and lung tissue changes induced by *M ramosissimus* and *T viride* spores following intratracheal inoculation in goats.

Materials and Methods

Animals—Thirty-six Boer-Spanish crossbred weanling goats of both sexes (18 females and 18 males) were used in the study. Males were castrated prior to purchase. Goats were housed in a 3-sided barn in 6 pens (6 goats [3 females and 3 males]/pen); the area of each pen was 17.7 m^2. Goats were administered medications to eliminate internal parasites[a] and coccidia.[b] Goats were limit-fed a commercial pelleted ration (44% grain concentrate, 20% alfalfa hay, 30% cottonseed hulls and meal, 5% molasses, vitamins A and E, and trace minerals); water was available ad libitum. The experimental protocol was approved by a regional animal care and use committee.

Experimental design—Goats were allowed to acclimate to their environment for 7 weeks. Each pen of 6 goats was randomly assigned as 1 of 4 treatment groups or 1 of 2 control groups. Two treatment groups received intratracheal inoculations with *M ramosissimus*, and 2 received intratracheal inoculations with *T viride*; in each pair of spore-treated groups, 1 group was exposed to dust and the other was not. Goats in a tent control group were used to evaluate the effect of confinement in a tent filled with aerosolized fine dust for 4 hours followed by intratracheal administration of 30 mL of physiologic saline (0.9% NaCl) solution. Goats in a pen control group were not exposed to dust or administered fungal or saline solution inoculations. All goats received IV injections of equine RBCs after the fourth dust exposure and immediately after their third fungal inoculation. The equine RBC injections were given to evaluate the ability of the study goats to generate antibodies against foreign antigens; this was done to determine that the goats were not immunosuppressed after dust and fungal treatments. Group (and pen) assignments were as follows: group 1, tent control group; group 2, *M ramosissimus* inoculation and dust exposure; group 3, *T viride* inoculation and dust exposure; group 4, *M ramosissimus* inoculation and no dust exposure; group 5, *T viride* inoculation and no dust exposure; and group 6, pen control group. Samples were collected immediately before dust and fungal exposure (designated as day 0 samples); after the first dust and fungal exposure, study days were numbered consecutively.

Preparation of equine RBCs for IV injection of goats—Blood was collected from a horse via jugular venipuncture into a 500-mL bottle containing 75 mL of acid citrate dextrose solution.[c] The plasma was removed by use of a sterile pipette following precipitation of the equine RBCs from the plasma. The equine RBCs were then washed in Alsever solution.[d] The equine RBC suspension was centrifuged at 430 X g for 30 minutes, and the process was repeated until the cells were washed 3 times in Alsever solution. The equine RBCs were stored in Alsever solution in the dark at 4°C. Prior to administration to the goats, the equine RBCs were washed 3 times with physiologic saline solution to remove the Alsever solution. The equine RBCs were counted in a hemacytometer, and 8.33×10^8 RBCs in a 4-mL volume were administered IV to each goat. Goats received only 1 injection of equine RBCs, which was administered after the fourth dust exposure and after the third intratracheal fungal inoculation.

Serum antibody assays—Of the 36 study goats, 12 received multiple intratracheal inoculations with *T viride*, and 12 received similar inoculations with *M ramosissimus*. All goats were injected IV with equine RBCs on day 40 of the experiment. A goat serum agglutination antibody assay was developed against *Trichoderma* spore antigen.[25] We were not successful in developing an agglutination assay against *Mucor* spore antigen. No agglutinating antibody was detected microscopically when postinoculation serum was mixed with a drop of spores on a microscope slide that was warmed to 37°C. *Trichoderma* spores (agglutinating antigen) were harvested from 25 Petri plates[e] (100 X 15 mm) containing malt extract agar. Each culture was incubated at 27°C for 8 days and then flooded with 10 mL of sterile physiologic saline solution. The fluid in the culture was mixed by swirling, and the spores were poured into a flask and allowed to settle for 20 minutes. The resulting spore suspensions (excluding the layer of settled spores) were used as the source of antigen for the assay. The spores were counted in a hemacytometer,[f] and a concentration of 2.09×10^6 spores/mL was determined. For each goat, a 2-fold serum dilution series (1:2 to 1:2,048) was made from serum collected on days 0, 7, 18, 32, 46, and 58 of the experiment. The serum dilutions (0.2 mL) were placed in a 24-well (flat bottom) plastic plate,[g] and 1 drop of spores from a Pasteur pipette[h] was added to each dilution well. The contents of the wells were mixed by rotating the plates by hand. Control wells contained fetal calf serum[i] or physiologic saline solution with 1 drop of antigen. The assay was incubated at 37°C for 30 minutes and assessed by use of an inverted microscope.[j]

A simple tube[k] agglutination assay was conducted[25] to evaluate antibody response to equine RBCs. A 2-fold dilution series of goat serum (1:2 to 1:2,048) was made for days 40, 46, 61, and 67 following equine RBC inoculation of goats on day 40. One drop of equine RBC hemagglutinin antigen (4% equine RBCs [optical density,[l] 0.2% transmitted light at 456 nm] diluted in physiologic saline solution) from a Pasteur pipette was placed in each dilution tube. Control tubes contained fetal calf serum or physiologic saline solution with 1 drop of equine RBCs. The hemagglutinin-antibody complexes were assessed visually.

Antigens used in the immunodiffusion assays were a pool of sonicated hyphae and spores from each fungus (*M ramosissimus* and *T viride*). Precipitating antibodies were determined by use of the Ouchterlony double immunodiffusion assay[26] involving Petri plates (100 X 15 mm) that were each filled with 30 mL of melted agarose[m] (1 g/99 mL of water). After the agarose solidified,

the wells were cut. The cut wells were aspirated, and 1 drop of melted agarose from a Pasteur pipette was placed in the bottom of each well to seal it. The agarose cutter[n] had 6 outer wells (internal diameter, 6 mm) and a center well (internal diameter, 9 mm); the wells were 8 mm apart. Goat sera were placed in the outer wells, and the sonicated[o] clarified fungal antigen was placed in the center well. Immunoprecipitin complex lines were evaluated with the aid of a microscope lamp[p] at 24, 48, and 72 hours after the inoculation of the Ouchterlony plates. Goat sera samples collected on days 0 and 52 were assayed by the method of Ouchterlony. Control wells contained fetal calf sera or PBS solution[q] with antigen.

The *Trichoderma* and *Mucor* preparations used as precipitating antigens were each grown on 15 Petri plates containing malt extract agar. Cultures were incubated at 27°C for 8 days until the plates were covered with hyphae and spores. Each culture plate was washed with 10 mL of PBS solution and scraped with a sterile cotton-tipped applicator[r]; the contents of the 15 plates for each fungal agent were pooled separately. Each pool was centrifuged[s] at 430 X *g* for 15 minutes to concentrate the fungal mass. Twenty-five milliliters of the fungal mass from each genus was sonicated at 400 W for 4 minutes with a 4-mm-diameter probe. The sonicated fungal mass (optical density, 0.2% transmitted light at 456 nm) was centrifuged at 2,000 X *g* for 30 minutes to clarify the antigen, and the supernatant was used as the precipitating antigen.

Antifungal IgG antibody ELISA—Differing concentrations of spores/mL (10^6 *M ramosissimus* spores/mL and 10^5 *T viride* spores/mL) in carbonate buffer solution[t] (pH, 9.0) were incubated overnight (approx 14 hours) in polystyrene immunoassay modules[u] at 4°C. The microtiter plates[v] were then washed 5 times with 300 µL of PBS solution with 0.05% Tween-20.[w] The plates were blocked with 300 µL of blocking buffer[x] for 1 hour at room temperature (approx 24°C) and then washed. The sera were assessed in 1:2 serial dilutions (1:50 to 1:3,200) with intact spores; to establish a background value, each sample was incubated without intact spores (1:50 dilution). These dilutions were incubated for 1 hour at room temperature, after which they were washed. Fifty microliters of horseradish peroxidase–conjugated rabbit anti-goat IgG[y] (secondary antibody, 1:10,000) and 50 mL of blocking buffer were added to each well, incubated for 1 hour at room temperature, and then washed. One hundred microliters of chromogenic substrate[z] was added to each well and allowed to incubate at room temperature for 10 minutes; the reaction was then stopped with 2N sulfuric acid.[aa] A plate reader[bb] with wavelength of 450 nm was used to analyze the plates. Results for dilutions were considered positive if the mean absorbance was 2 SDs greater than the background value for each sample. Two treatment groups (groups 2 and 3) were examined for antifungal IgG antibodies. Goat sera collected prior to dust and fungal inoculations were used as control samples in the assay.

Administration of aerosolized dust to goats—The feedyard dust preparation has been previously characterized and described.[13] Coarse dust was submitted to a company[cc] that was capable of grinding feedyard dust into fine particles; particles were sized via dry dispersion. Four 1-g samples were each passed through an instrument[dd] to determine the mean particle size distribution. The dust was autoclaved[ee] for 15 minutes at 121°C and a pressure of 6.9 kPa to kill the microbes (bacteria, fungi, and their spores) that are typically present in feedyard dust.[14] Prior to autoclaving, the dust contained 21.28 µg of endotoxin/g of dust, and after sterilization it contained 10.78 µg of endotoxin/g of dust. Microbial cultures of autoclaved dust yielded no viable colonies of bacteria or fungi.

Briefly, the dust was administered to goats confined in a semi–air-tight tent.[ff] The drop rate was 35 g of dust evenly distributed during each 15-minute period by continuously feeding the dust directly into the funnel that led to a jet mill.[gg] Dust was disseminated over a period of 4 hours, and goats were exposed to 600 g of aerosolized fine dust inside the tent. The aerosolization process was previously described in detail.[12,13] Goats underwent 7 dust exposures (on days –7, 0, 17, 31, 45, 58, and 67). The first dust exposure was given to evaluate the endotoxin response; then approximately every other week, a dust exposure was performed and followed by fungal spore inoculation. The seventh dust exposure was given 24 hours prior to necropsy on day 68. The 4-hour dust exposure also preceded each saline solution inoculation of the tent control goats.

As in previous experiments,[8,12,13] dust samples were collected in the tent to determine the amount of dust that settled from the air. A 5-stage cyclone device[hh] attached to a vacuum pump[ii] (suction rate, 28.4 L/min) was used to collect fine dust particles (5.2 to 0.32 µm in diameter) during each 4-hour exposure.

Preparation of fungal spores—Each fungus (*M ramosissimus* and *T viride*)[jj] was grown on 60 malt extract agar[kk] plates (100 X 15 mm) at 28°C for a period (5 to 14 days) that was sufficient to allow massive sporulation. Preparation of spores has been described in detail.[8] Briefly, spores were separated from hyphae by use of a sieve and were counted in a hemacytometer. Spore suspensions were titrated in triplicate on malt extract agar Petri plates. Spore preparations for each fungus were mixed and divided equally into twelve 30-mL syringes.

Intratracheal inoculation of fungal spores—Contents of the syringes containing fungal spores were mixed thoroughly immediately prior to intratracheal inoculation. Goats in the 4 spore treatment groups (2 *M ramosissimus*–inoculated and 2 *T viride*–inoculated groups) and the tent control group were anesthetized. Each goat was administered atropine sulfate[ll] (0.04 mg/kg, IM) followed by an IV injection of a combination of butorphanol[mm][nn] (0.5 mg/kg) and ketamine hydrochloride[nn] (4.4 mg/kg). As the goats' lung function decreased because of pathologic changes during the study, the doses of butorphanol and ketamine administered were decreased by 25%. A speculum[oo] with a light source was inserted into the oral pharynx of the goat, and the epiglottis was viewed. A 1-mL pipette[pp] equipped with a tip consisting of a hollow rubber tube was inserted past the epiglottis into the proximal portion of the trachea. A syringe containing the spore solution was attached to the pipette, and 30 mL of the spore preparation was

inoculated into the trachea. Each spore type was inoculated every other week (on days 0, 17, 31, 45, and 58) until 5 treatments had been administered. Goats in the tent control group were each similarly anesthetized and inoculated via the trachea with 30 mL of physiologic saline solution.

Collection of data and blood samples—The first dust treatment was specifically used to evaluate the endotoxin reaction following dust application. Rectal temperatures[qq] were measured immediately before the dust exposure (0 hours) and at 4, 8, 12, 24, 48, and 72 hours after completion of the 4-hour dust exposure; at the same time points, a blood sample (10 mL) was collected in a tube containing anticoagulant (EDTA)[rr] for total WBC counts.[ss] Thereafter, following each dust exposure and fungal inoculation, rectal temperature was measured at 4 hours and then daily for 3 days. White blood cell counts were assessed 48 hours following each fungal inoculation (ie, on days 19, 33, 47, and 60) and on day 67. Serum samples were collected for antibody titer determinations on days 0, 7, 18, 32, 46, and 58. During the study period, average daily gains among goats in each group were assessed for comparison.

Necropsies—At the completion of the study (day 68), all goats were euthanatized 12 hours after the seventh dust treatment via IV administration of an overdose of a barbiturate solution.[tt] All gross necropsies were performed by a veterinarian under the supervision of a veterinary pathologist (JRA). Histologic examinations of the tissues were performed by the pathologist, who was not aware of the group status of each goat.

During necropsy of each goat, sterile cotton-tipped applicators were used to collect mucus and tissue fluid (0.1 mL) from the thoracic cavity, proximal and distal portions of the trachea, a bronchus, and lungs. Mucus and tissue fluid were expressed by rotating the swab onto the surface of a malt extract agar plate and were further dispersed on the surface of the agar by use of the streak dilution technique. The inoculated Petri plates were incubated[uu] at 28°C. Fungal colonies on incubated plates were enumerated 24, 72, and 120 hours after onset of incubation.

In addition, a limited number of goats were used to quantify the number of fungal CFUs from consolidated lung lesions. Ten necropsied goats (3 in group 2, 3 in group 3, 2 in group 4, and 2 in group 5) were selected, and a weighed sample (1 g)[vv] from a major consolidated lung lesion was aseptically collected from each animal. The lesion sample was ground in a tissue grinder,[ww] and a series of 10-fold dilutions were made in triplicate. A 0.1-mL aliquot from each dilution was plated onto a Petri plate of malt extract agar to determine the number of fungal CFUs per gram.

Tissue samples from all 6 lung lobes of each goat were immediately placed in neutral-buffered 10% formalin.[xx] The tissues were processed and stained[27] with H&E[yy] and Gomori's methenamine silver stain.[zz]

Statistical analysis—Mean values for measured variables were compared by use of an ANOVA.[28] Mean values for rectal temperature and total WBC counts were compared between treatment and control groups throughout the study and within collection periods for specific study days. Geometric means (natural logarithms) were determined for serum antibody titers. Significant differences between mean values were determined by the use of the Bonferroni and Dunnett adjusted paired *t* test, which allowed pairwise comparisons of means between groups within any day of sample collection. For all assessments, a value of $P \leq 0.05$ was considered significant.

Results

Total and viable spore counts—For each exposure of the 2 fungi, mean counts of cultured viable spores were subtracted from the mean counts of total spores to determine the mean number of nonviable spores. The mean value for each 30-mL spore suspension of the same spore type was calculated for all treatments, and the mean values for each goat per group were used to calculate the overall mean ± SEM total spore counts, mean viable spore counts, and mean nonviable spore counts for each fungal type per 30-mL dose. For *M ramosissimus*, the mean ± SEM spores/30-mL dose for the 5 inoculations was $2.91 \times 10^{10} \pm 1.55 \times 10^{9}$, which included $7.04 \times 10^{8} \pm 1.61 \times 10^{8}$ viable spores and $2.84 \times 10^{10} \pm 1.46 \times 10^{9}$ nonviable spores. For *T viride*, the mean ± SEM spores/30-mL dose for the 5 inoculations was $1.48 \times 10^{11} \pm 2.59 \times 10^{10}$, which included $1.98 \times 10^{9} \pm 7.05 \times 10^{8}$ viable spores and $1.46 \times 10^{10} \pm 2.61 \times 10^{10}$ nonviable spores.

Characterization of feedyard dust on the basis of size distribution—Four histograms representing the size of the dust particles were extremely similar (data not shown). The mean ± SD particle diameter was ≤ 7.72 ± 0.69 µm, and each histogram indicated a biphasic pattern. The first phase contained particles < 1 µm in diameter, and the second phase contained particles < 10 µm in diameter with a rapid cutoff (skewed to the left).

Aerosolized dust in the tent—The mean ± SEM quantity of dust collected in the Petri plates after seven 4-hour dust exposures was indicative of 25.3 ± 2.08 g/m² of settled dust from the tent air. The mean total dust collected by the cyclone device over seven 4-hour dust exposures was 2.34 ± 0.007 g/m³.

Endotoxin effects of dust on goats—In the 18 goats that were exposed to dust inhalation, the first dust exposure induced a significant ($P \leq 0.001$) increase in mean ± SEM rectal temperature after 4 (39.24 ± 0.08°C), 8 (39.41 ± 0.09°C), and 12 (39.42 ± 0.06°C) hours, compared with the value at 0 hours (38.67 ± 0.06°C); at 24 and 72 hours, mean rectal temperature did not differ from 0 hours. The mean ± SEM overall WBC count was significantly ($P \leq 0.001$) greater for groups 2 and 3 (19,422 ± 418 cells/µL), compared with the overall value for groups 4 and 5 (17,404 ± 365 cells/µL); there was no significant difference in WBC count between sexes. Nevertheless, among goats in groups 2 and 3, there was a gradual increase in mean total WBC count following the first dust treatment from 0 hours (19,064 ± 908 cells/µL) through 4 hours (19,825 ± 987 cells/µL) and 8 hours (22,019 ± 830 cells/µL) followed by decreases at 12 hours (19,703 ± 936 cells/µL),

24 hours (18,346 ± 1,069 cells/μL), and 72 hours (17,556 ± 1,184 cells/μL). The increase in WBC count for groups 2 and 3 initially was caused by the endotoxin in the dust.

Overall effects on goats—Mean average daily gains in the tent and pen control groups were not significantly different and values were not significantly different between *M ramosissimus*-inoculated groups 2 and 4; also, values were not significantly different between *T viride*-inoculated groups 3 and 5. Therefore, those values were combined into 3 respective groups for analysis. The average daily gain was significantly ($P < 0.001$) different among the combined groups; the combined value for the *T viride*–inoculated groups (0.07 kg/d) was greater than the combined value for the *M ramosissimus*–inoculated groups (–0.07 kg/d) but was not different from the combined control group value (0.10 kg/d).

Seven goats died during the study, of which 2 were in group 2 (deaths on days 32 and 45), 3 were in group 3 (deaths on days 18, 18, and 45), and 2 were in group 4 (deaths on days 49 and 62). One goat in group 2 was euthanatized on day 32, and 2 goats in group 4 were found dead on days 49 and 62. Four goats did not recover from anesthesia (3 goats in group 3 on days 18, 18, and 45 and 1 goat in group 4 on day 49).

Responses to dust exposure and intratracheal spore inoculations—Goats responded calmly to the five 4-hour dust exposures. Goats were covered with

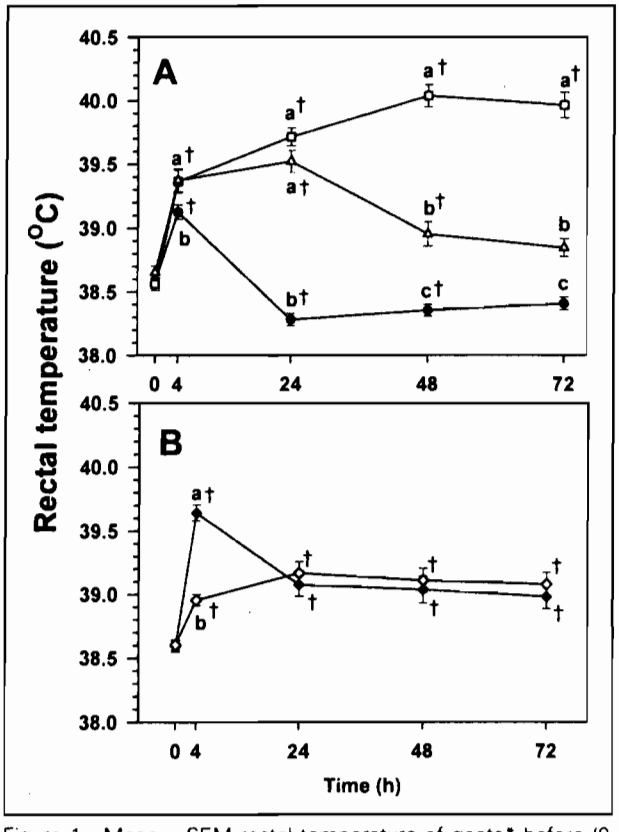

Figure 1—Mean ± SEM rectal temperature of goats* before (0 hours) and at intervals after intratracheal inoculation with *Mucor ramosissimus* spores, *Trichoderma viride* spores, or saline (0.9% NaCl) solution and exposure or nonexposure to feedyard dust. Depending on treatment group allocation, goats were (or were not) exposed to aerosolized sterile dust for 4 hours followed by intratracheal inoculation with 30 mL of a fungal spore preparation or saline solution; datum points are the mean values calculated from 5 dust exposures and fungal inoculations for each group. Treatment group data were combined for comparison of rectal temperatures in goats that underwent *M ramosissimus* inoculation (with or without dust exposure; n = 12; white squares), goats that underwent *T viride* inoculation (with or without dust exposure; 12; white triangles), and goats that underwent saline solution inoculation with dust exposure or no inoculation and no dust exposure (12; black circles; A) and for comparison of values in goats that were (18; black diamonds) and were not (18; white diamonds) exposed to feedyard dust (B). *Seven goats died or were euthanatized during the experiment; the deaths occurred between days 18 and 62, and the consolidated lung lesions in these animals were included with those in animals that completed the study on day 68. Of those goats that died during the study, 3 were *T viride* inoculated and exposed to dust (2 deaths occurred on day 18, and 1 death occurred on day 45), 2 were *M ramosissimus* inoculated and exposed to dust (deaths occurred on days 32 and 45), and 2 were *M ramosissimus* inoculated and not exposed to dust (deaths occurred on days 49 and 62). †Value is significantly ($P < 0.05$) different from the value at 0 hours (Dunnett *t* test). a-cAt a specific time point, values with different letters are significantly ($P < 0.05$) different (Bonferroni pairwise comparison).

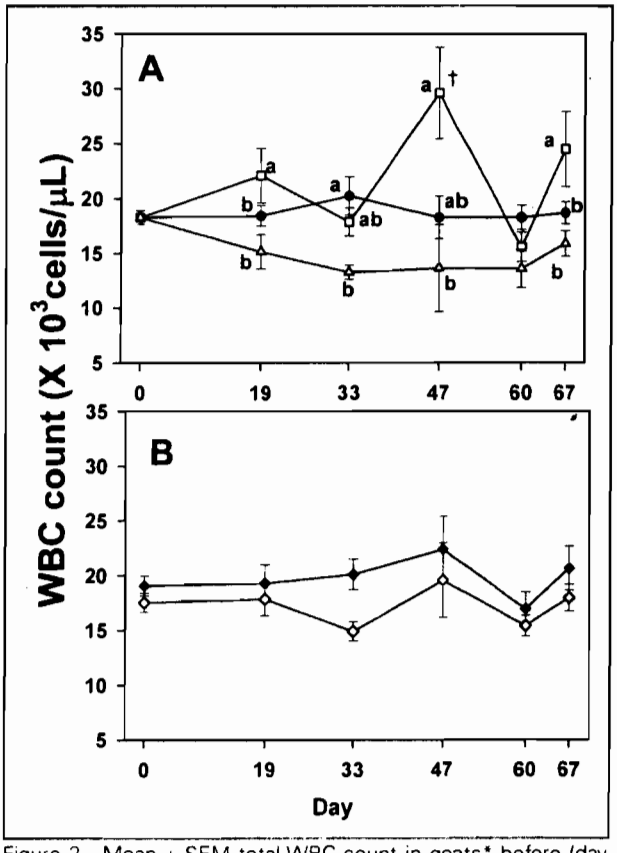

Figure 2—Mean ± SEM total WBC count in goats* before (day 0) and at days 19, 33, 47, 60 and 67 in a study of the effects of multiple intratracheal inoculations with *M ramosissimus* spores, *T viride* spores, or saline (0.9% NaCl) solution and exposures or nonexposures to feedyard dust. Dust exposures were performed on days –7, 0, 17, 31, 45, 58, and 67, and fungal inoculations were performed on days 0, 17, 31, 45, and 58. Treatment group data were combined for comparison of total WBC counts in goats that underwent *M ramosissimus* inoculation (with or without dust exposure; n = 12; white squares), goats that underwent *T viride* inoculation (with or without dust exposure; 12; white triangles), and goats that underwent saline solution inoculation with dust exposure or no inoculation and no dust exposure (12; black circles; A) and for comparison of values in goats that were (18; black diamonds) and were not (18; white diamonds) exposed to feedyard dust (B). *See* Figure 1 for remainder of key.

dust when they were removed from the tent after a 4-hour exposure. The internal nares were covered with dust, which became moistened by vapor from respiration. Intermittent coughing was evident after the goats were removed from the tent.

From data collected after each of 5 fungal or saline solution inoculations, mean rectal temperature in the *M ramosissimus*–inoculated, *T viride*–inoculated, and control groups (like treatments combined) differed significantly ($P < 0.001$) during the 3-day period following fungal inoculations (Figure 1). Assessment (Bonferroni *t* test) of mean rectal temperature values for the entire 3-day period after fungal or saline solution inoculations indicated that goats in the *M ramosissimus*–inoculated groups (groups 2 and 4; n = 12) had the highest overall mean rectal temperature (39.5 ± 0.05°C) and that goats in the control groups (groups 1 and 6; 12) had the lowest overall mean rectal temperature (38.6 ± 0.03°C). Overall mean rectal temperature in the *T viride*–inoculated groups (groups 3 and 5; 12) was 39.1 ± 0.04°C. Rectal temperatures in male (n = 18) and female (18) goats did not differ significantly. Results of the Bonferroni paired *t* test indicated no significant differences in mean rectal temperature between the control groups (groups 1 and 6), between the *M ramosissimus*–inoculated groups with or without dust exposure (groups 2 and 4, respectively), or between the *T viride*–inoculated groups with or without dust exposure (groups 3 and 5, respectively) at any time point. The Dunnett paired *t* test revealed that the mean rectal temperature for the pen control group was significantly ($P \leq 0.05$) less than the value for all other treatment groups at 4 hours after intratracheal inoculations. For the pen control group, there were also significant ($P < 0.001$) differences among the time points at which the measurements were made; the lowest value was recorded at 0 hours (38.6 ± 0.03°C), the highest value was recorded at 4 hours (39.2 ± 0.05°C), and intermediate values were recorded at 24, 48, and 72 hours.

With regard to mean rectal temperature, significant group or pen differences were detected. Among the 6 groups, the lowest mean rectal temperatures were recorded for control groups 1 and 6 and the highest mean rectal temperatures were recorded for the *M ramosissimus*-inoculated groups with or without dust exposure (groups 2 and 4, respectively). The *T viride*-inoculated groups with or without dust exposure (groups 3 and 5, respectively) had intermediate values.

With regard to mean WBC counts, significant group or pen differences were detected. Mean WBC counts were highest for groups 1 and 4, and values were lowest for group 5. Mean WBC counts for groups 3, 5, and 6 were significantly lower than findings for groups 1 and 4. Mean WBC count for group 2 (the *M ramosissimus*–inoculated group with dust exposure) was not different from the value for the tent control group or the *M ramosissimus*–inoculated group without dust exposure (groups 1 and 4, respectively).

The overall mean total WBC count was significantly ($P \leq 0.004$) higher in the dust-exposed groups (19,752 ± 754 cells/μL), compared with nonexposed groups (17,181 ± 707 cells/μL) and significantly ($P \leq 0.026$) different among time points. Prior to the first dust exposure and fungal or saline solution inoculation, mean WBC count in all 6 groups was (18,290 ± 625 cells/μL). The WBC count following the third dust exposure and fungal or saline solution inoculation (20,896 ± 2,261 cells/μL) was the highest, and the value following the fourth dust exposure and fungal or saline solution inoculation (16,044 ± 844 cells/μL) was the lowest. Intermediate values were detected following the first, second, and fifth dust exposures and fungal or saline solution inoculations. By use of the Dunnett paired *t* test, a significant ($P \leq 0.05$) difference in mean

Table 1—Mean ± SEM size (mm^3) of consolidated lesions in groups* of 6 goats† that underwent multiple intratracheal inoculations with *Mucor ramosissimus* spores, *Trichoderma viride* spores, or saline (0.9% NaCl) solution and were or were not exposed to feedyard dust.

	Group					
Lung lobe	**1**	**2**	**3**	**4**	**5**	**6**
Right						
Cranial	0	100,802 ± 41,486	7,194 ± 3,210	82,667 ± 19,343	24,626 ± 14,188	0
Middle	0	28,151 ± 9,074	3,270 ± 2,175	37,391 ± 8,491	25,843 ± 6,308	0
Caudal	0	177,063 ± 60,332	1,679 ± 1,679	121,460 ± 21,210	14,482 ± 6,310	0
Left						
Cranial	0	13,859 ± 7,100	4,800 ± 1,916	33,930 ± 13,347	125 ± 125	0
Middle	0	25,666 ± 5,862	7,645 ± 1,829	43,411 ± 18,346	9,203 ± 4,288	0
Caudal	0	71,543 ± 24,272	4,092 ± 4,092	86,600 ± 9,815	6,240 ± 6,240	0

Values represent total mean size (mm^3 [width × length × thickness]) of consolidated lesions in each group. Depending on treatment group allocation, goats were (or were not) exposed to aerosolized sterile dust for 4 hours followed by intratracheal inoculation with 30 mL of a fungal spore preparation or saline solution; there were 7 dust exposures and 5 fungal or saline solution inoculations. *Group treatments were as follows: group 1, tent control group (dust exposure and saline solution inoculation); group 2, *M ramosissimus* inoculation and dust exposure; group 3, *T viride* inoculation and dust exposure; group 4, *M ramosissimus* inoculation and no dust exposure; group 5, *T viride* inoculation and no dust exposure; and group 6, pen control group (no inoculation and no dust exposure). †There were 7 goats that died or were euthanatized during the experiment; the deaths occurred between days 18 and 62, and the consolidated lung lesions in these animals were included with those in animals that completed the study on day 68. Of those goats that died earlier in the study, 3 were in group 3 (2 deaths occurred on day 18, and 1 death occurred on day 45), 2 were in group 2 (deaths occurred on days 32 and 45), and 2 were in group 4 (deaths occurred on days 49 and 62).

Table 2—Mean ± SEM size (mm^2) of atelectatic lesions in groups* of 6 goats† that underwent multiple intratracheal inoculations with *M ramosissimus* spores, *T viride* spores, or saline solution and were or were not exposed to feedyard dust.

	Group					
Lung lobe	**1**	**2**	**3**	**4**	**5**	**6**
Right						
Cranial	884 ± 557	782 ± 782	0	0	132 ± 86	138 ± 91
Middle	291 ± 182	488 ± 488	152 ± 118	0	49 ± 43	17 ± 13
Caudal	8 ± 8	0	60 ± 60	0	88 ± 88	0
Left						
Cranial	8 ± 8	89 ± 89	125 ± 81	0	400 ± 381	1 ± 1
Middle	115 ± 51	0	0	0	25 ± 25	4 ± 4
Caudal	9 ± 9	0	35 ± 24	0	934 ± 788	0

Values represent total mean size (mm^2 [length × width]) of atelectatic lesions in each group. Seven goats died or were euthanatized during the study, and the data for these animals were included with those from animals that completed the study on day 68.

See Table 1 for key.

total WBC count was identified between the *M ramosissimus*–inoculated groups (21,869 ± 1,397 cells/μL) and the *T viride*–inoculated groups (14,320 ± 940 cells/μL); however, the value for the control groups (18,778 ± 616 cells/μL) was not different from that of the *T viride*–inoculated groups (**Figure 2**).

Consolidated lesions were confined to the *M ramosissimus*–inoculated and *T viride*–inoculated goats (**Table 1**). Mild atelectasis was evident in most groups, including the control groups (**Table 2**). The overall mean extent of consolidated lesions for both lungs in the *M ramosissimus*–inoculated groups was significantly ($P \leq 0.001$) higher (59,321 mm^3) than that of the control groups (0 mm^3). The overall mean extent of consolidated lesions for both lungs in the *T viride*–inoculated groups (7,800 mm^3) was not significantly ($P \leq 0.06$) different than that of the control groups because of the high degree of variability in the sizes of the lesions. The mean extent of consolidated lesions located in the different lobes of the lungs were compared; the value for all lobes of the *M ramosissimus*–inoculated goats (415,246 ± 70,791 mm^3) and the value for the middle lobes of both lungs of the *T viride*–inoculated goats (11,490 ± 2,606 mm^3) were each significantly ($P \leq 0.001$; ANOVA) larger than the values for all lobes (0 mm^3) and the middle lobes of both lungs (0 mm^3) of the control goats.

The ventral portion of the lungs was affected most in the goats that were exposed to dust and inoculated with fungal spores; this finding was most notable in the goats that were exposed to dust and inoculated with *M ramosissimus*. The lesions induced by inoculation with *M ramosissimus* were more severe and were hard, uniform, and consolidated, compared with lesions in goats that were inoculated with *T viride*. The lesions observed in the *T viride*–inoculated goats were larger and softer than those in *M ramosissimus*–inoculated goats. However, 3 of the *T viride*–inoculated goats had notably small lesions.

For spore-treated goats, the total combined extent of consolidated lesions of the right lung were significantly ($P = 0.006$) greater than the extent of those found in the left lung (26,357 ± 4,934 mm^3 vs 17,062 ± 3,084 mm^3). The total combined extent of consolidated lesions in both lungs of spore-treated goats (compared by lobe) was 40,263 ± 8,661 mm^3 in the caudal lobes, 22,334 ± 5,495 mm^3 in the cranial lobes, and 15,048 ± 2,608 mm^3 in the middle lobes.

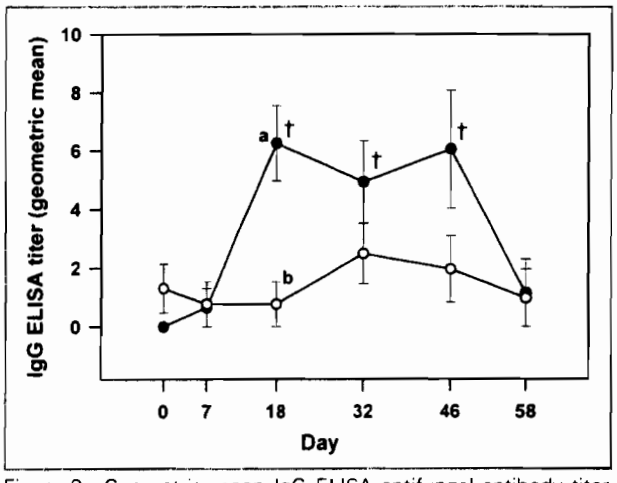

Figure 3—Geometric mean IgG ELISA antifungal antibody titer in goats* before (day 0) and at days 7, 18, 32, 46, and 58 in a study of the effects of multiple intratracheal inoculations with *M ramosissimus* spores, *T viride* spores, or saline (0.9% NaCl) solution and exposures or nonexposures to feedyard dust. Dust exposures were performed on days –7, 0, 17, 31, 45, 58, and 67, and fungal inoculations were performed on days 0, 17, 31, 45, and 58. Treatment group data were combined for comparison of geometric mean titers in goats that underwent *M ramosissimus* inoculation (with or without dust exposure; n = 12; black circles) and goats that underwent *T viride* inoculation (with or without dust exposure; 12; white circles). *See* Figure 1 for remainder of key.

Histologic examination of lung tissue from spore-treated goats revealed patchy to coalescing alveolar and airway exudation of fibrin, neutrophils, and macrophages. Fibrosis with formation of multiple granulomas involving Langhans-type multinucleate giant cells was evident. Degenerative fungal forms (including rudimentary germ-tube formation from spores) were present in some granuloma nidi. By use of modified Gomori methenamine silver stain, fungal spores but no hyphae were detected in all sections of lung tissue examined. Multifocal mineralization was present in some of the granulomas of the *M ramosissimus*–inoculated goats. Also, there was an increase in the amount of fibrosis in the lungs of the *M ramosissimus*–inoculated goats, compared with findings in the lungs of the *T viride*–in-

Table 3—Fungal isolates cultured from various tissues obtained from groups* of 6 goats† that underwent multiple intratracheal inoculations with *M ramosissimus* spores, *T viride* spores, or saline solution and were or were not exposed to feedyard dust.

Specimen	Group 1	2	3	4	5	6
Mesenteric lymph node	0	1 (M)	6 (T)	0 (M)	6 (T)	0
Thoracic cavity fluid	0	0 (M)	3 (T)	0 (M)	0 (T)	0
Trachea						
Proximal portion	1 (P), 1 (M)	5 (M)	6 (T)	4 (M)	3 (T), 1 (M)	0
Distal portion	0	4 (M)	6 (T)	3 (M)	4 (T)	0
Bronchus	0	5 (M)	4 (T)	3 (M)	3 (T)	0
Lung lobe						
Left						
Cranial	0	6 (M)	2 (T)	3 (M)	1 (T)	0
Middle	0	4 (M)	2 (T)	5 (M)	5 (T)	0
Caudal	0	6 (M)	4 (T)	4 (M)	2 (T)	0
Right						
Cranial	0	5 (M)	6 (T)	3 (M)	5 (T)	0
Middle	0	5 (M)	5 (T)	3 (M)	6 (T)	0
Caudal	0	6 (M)	3 (T)	3 (M)	6 (T)	0
Liver	0	1 (M)	3 (T)	1 (M)	0 (T)	0
Spleen	0	0 (M)	2 (T)	0 (M)	0 (T)	0
Kidney	0	0 (M)	0 (T)	0 (M)	0 (T)	0

Samples were obtained 24 hours after the last exposure to aerosolized sterile feedyard dust and 10 days following the last intratracheal inoculation with fungal spore suspension or saline solution. Values indicate the number of goats for which culture results were positive for a specific fungus; the letter in parentheses indicates the type of fungus isolated (M = *M ramosissimus*. T = *T viride*. P = *Penicillin* contaminant). Seven goats died or were euthanatized during the study, and the data for these animals were included with those from animals that completed the study on day 68.

See Table 1 for key.

oculated goats. Among the 4 spore-treated groups, no histologically distinctive lung changes were identified between goats that were exposed to dust and those that were not exposed to dust. Gomori methenamine silver stain–positive material of unknown origin was frequently present in macrophages in all lymph nodes examined. Patchy atelectasis was seen in some sections of lung tissue from the goats in control groups 1 and 6. No fungal spores were detected in any tissues collected from the control goats, and no Gomori methenamine silver stain–positive macrophages were identified in any lymph nodes examined. No histologically distinctive lung changes were identified between goats in the tent control group that underwent dust exposure and saline solution inoculation and those in the pen control group that were not exposed to dust and not inoculated.

Tube agglutination titers of antibody against equine RBCs—With regard to geometric mean agglutinating antibody titer responses, there were no significant differences among the goat groups. However, there were significant differences ($P < 0.001$) among time points; compared with the geometric mean titer on day 0 (0 ± 0), the value was increased at day 6 (4.29 ± 0.19) and day 21 (5.25 ± 0.15) with a subsequent decrease on day 27 (4.49 ± 0.15).

Agglutinating antifungal antibody titers—We were unable to detect agglutinating antibodies against spores of *M ramosissimus*. Agglutinating antibodies against *T viride* spores were detected in sera collected on day 0; however, these titers did not exceed a 1:16 dilution. Goats that underwent intratracheal inoculation with *T viride* developed agglutination titers of 1:1,024. The geometric mean agglutinating antifungal antibody titer among the *T viride*–inoculated goats was significantly ($P < 0.001$) larger than that of the control goats (4.91 ± 0.29 vs 2.54 ± 0.08). Values in dust-exposed and nonexposed goats did not differ significantly. The geometric mean antifungal antibody titers at days 24, 38, 52, and 65 (3.55 ± 0.26, 4.11 ± 0.41, 3.88 ± 0.44, and 4.17 ± 0.49, respectively) differed significantly ($P < 0.001$) from the value at day 0 (2.33 ± 0.21).

Fungus-induced IgG antibody—The overall geometric mean IgG antibody response for groups 2 and 4 (goats that underwent *M ramosissimus* inoculation with and without dust exposure) was significantly higher than the response for groups 3 and 5 (goats that underwent *T viride* inoculation with and without dust exposure; 3.066 ± 0.65 and 1.33 ± 0.35, respectively). The geometric mean IgG antibody titer among the *M ramosissimus*–inoculated goats was significantly greater than the value among the *T viride*–inoculated goats on day 24 (**Figure 3**). Furthermore, the geometric mean IgG antibody titer among the *M ramosissimus*–inoculated goats was significantly greater than the value at day 0 on days 24, 38, and 52.

Precipitating antibodies—After 24 hours of incubation of the *M ramosissimus*–specific Ouchterlony immunodiffusion plates, 2 sharply defined immunoprecipitating (antigen-antibody complex) lines developed between the center well containing the *M ramosissimus* antigens and the outer wells that contained the goat *M ramosissimus* antisera collected on days 24, 38, 52, and 65. No immunoprecipitin lines developed with serum collected on day 0 from any of the goats. On plates containing *T viride* antisera collected on the same days and sonicated *T viride* antigen, similar immunoprecipitin patterns developed. No immunoprecipitin lines developed when sera collected from the tent or pen

control goats on the same days were evaluated against either fungal antigen (data not shown).

Fungal isolations from the respiratory tract and organ tissues—*Mucor ramosissimus* and *T viride* were isolated from various specimens collected from goats in the study groups (**Table 3**). The numbers of fungal CFUs derived from consolidated lung tissue were determined. In groups 2 and 3 (goats that underwent fungal inoculation with dust exposure), there was $8.03 \times 10^5 \pm 2.78 \times 10^5$ CFUs/g of lung lesions, which was significantly ($P < 0.001$) greater than the value in groups 4 and 5 (goats that underwent fungal inoculation without dust exposure; $1.16 \times 10^4 \pm 2.15 \times 10^4$ CFUs/g of lung lesions). The number of fungal CFUs derived from consolidated lung tissue in the *T viride*–inoculated goats ($5.55 \times 10^5 \pm 4.13 \times 10^5$ CFUs/g of lung lesions) was not significantly ($P < 0.06$) different from the value in the *M ramosissimus*–inoculated goats ($5.01 \times 10^5 \pm 3.76 \times 10^5$ CFUs/g of lung lesions).

Discussion

In the present study, the goats developed a typical endotoxin-induced inflammatory response following the initial sterile fine dust exposure; this exposure was performed prior to fungal inoculations, thereby eliminating any confounding effect of fungal treatment on rectal temperature and total WBC count. Compared with the value before dust exposure, a significant increase in rectal temperature was detected at 4, 8, and 12 hours. The mean overall WBC count for the 18 goats that were exposed to dust was significantly increased, compared with the count for the 18 goats that were not exposed to dust. This increase in rectal temperature and total WBC count was previously observed in goats[14] and sheep[13] after exposure to coarse sterile dust[8] and coarse nonsterilized dust. Endotoxin is relatively resistant to autoclave temperatures applied for 15 minutes because only approximately half of the initial endotoxin concentration was destroyed under the conditions of the present study.

Systemic fungal diseases can be divided into 2 groups: diseases that develop only in immunocompromised individuals (group 1) and those that develop in previously healthy individuals (group 2). Diseases in group 1 include systemic candidiasis, pneumocystosis, aspergillosis, and mucormycosis; diseases in group 2 include paracoccidioidomycosis, coccidioidomycosis, histoplasmosis, blastomycosis, and cryptococcosis. All of these diseases have the capacity for lung involvement. The diseases in group 1 have worldwide distributions, whereas the diseases in group 2 are typically associated with specific geographic locations. Cellular immunity is a critical component of a body's ability to prevent development of the diseases in both groups 1 and 2. As long as an individual's cellular immunity remains highly competent, it is unlikely (except in diseases like histoplasmosis and blastomycosis) that the aforementioned fungal lung infections would develop. Nevertheless, such fungal diseases do develop, and many of the reasons why are unknown.[29] In the present study, one of the fungi from group 1, *M ramosissimus*, was used and was capable of causing pathologic processes in immunocompetent goats. The inhalation of fine dust may have been a contributing factor in progression of those pathologic processes.

As is typical of inhalation pneumonia, the ventral portion of each lung in the goats of the present study was primarily affected. In our study and previous experiments,[8] this distribution was most noticeable in the *M ramosissimus*–inoculated goats. Progression of the lesions appeared to extend from the middle and cranial lobes into the caudal lobe.[30] The *M ramosissimus*–inoculated and *T viride*–inoculated goats that were exposed to feedyard dust appeared to be more severely affected early in the study period, compared with the *M ramosissimus*–inoculated and *T viride*–inoculated goats that were not exposed to feedyard dust; however, that difference disappeared with time. As the extent of the consolidated lesions increased, there was a greater risk of anesthesia-associated death; therefore, the doses of both butorphanol and ketamine were decreased by a fourth.

Atelectatic lesions in the lungs of the goats were of interest because the tent control group (which was exposed to dust) had larger atelectatic lesions of the right cranial lung lobe (884 ± 557 mm^2), compared with lesions in that lung region of goats that were *M ramosissimus* inoculated but not exposed to dust (group 4; 0 mm^2), those that were *T viride* inoculated but not exposed to dust (group 5; 132 ± 86 mm^2), and those that were not inoculated and not exposed to dust (pen control group; 138 ± 91 mm^2). The goats were assigned to their study group via random number selection. The atelectatic lesions did not occur by chance in the various groups, and it appears that those lesions developed, at least in part, because of the dust exposure. The goats of group 5 (those that underwent *T viride* inoculation and no dust exposure) had rather large atelectatic lesions (400 ± 381 mm^2). One could argue that the atelectatic lesions were present prior to the dust exposures, but again, this appears unlikely because of the distribution of atelectatic lesions. During periods of dust exposure, groups of goats that were not to be exposed to dust were moved to an outside pen as far away from the dust exposure area as possible to avoid cross contamination of dust particles.

It is interesting that both *M ramosissimus* and *T viride* were not killed by the goats' immune systems for 10 days following the final (fifth) intratracheal fungal inoculations. The viable spores were present in the consolidated lesions in great numbers: 5.55×10^5 spores/g of tissue for *T viride* and 5.01×10^5 spores/g of tissue for *M ramosissimus*. Nevertheless, the body defenses appeared to prevent these viable spores from germinating because no hyphae were detected in the lung tissues via histologic examination. On rare occasions, rudimentary germ-tube formation from spores in the tissues of these immunocompetent goats was detected. Spores were quantified in 1 g of lung lesion tissue from 10 goats, and significantly more spores were cultured from 5 dust-exposed goats, compared with the number cultured from 5 nonexposed goats. This appears to indicate that feedyard dust does have an effect on the immune-mediated destruction of the spores, perhaps prior to their entrapment in a consolidated lesion.

The spores did not develop hyphae; therefore, the presence of significantly more spores in lung lesion tissue of the dust-exposed goats, compared with the number of spores in nonexposed goats, is indicative of immune system suppression. However, there were no significant differences in agglutinating antibody titers against equine RBCs between the control groups and the spore-treated groups (those that were or were not exposed to dust). There was no significant difference in agglutinating antibody titers between *T viride*–inoculated goats that were or were not exposed to dust and no significant difference in ELISA IgG antifungal titers between the *M ramosissimus*–inoculated goats that were or were not exposed to dust. However, the *M ramosissimus*–inoculated goats had significantly higher ELISA geometric mean titers, compared with the *T viride*–inoculated goats. We were unable to detect anti-*M ramosissimus* agglutinating antibodies in sera collected from goats of the present study.

In *M ramosissimus*–inoculated and *T viride*–inoculated goats that were or were not exposed to feedyard dust, genus-specific precipitating antibody was induced following multiple intratracheal fungal exposures, and no cross-reactive antifungal antibodies were detected. We were surprised that no *M ramosissimus*– or *T viride*–inoculated goats had precipitating antibodies against these common feedyard fungi on day 0. Also, none of the control goats (tent and pen control groups) had any cross-reactive precipitating antibodies against either fungus. The goats exposed to *M ramosissimus* or *T viride* appeared to be immunocompetent on the basis of their ability to synthesize hemagglutinating, agglutinating, and precipitating antibodies, similar to those detected in goats that were not inoculated with a fungus.

In the present study, *M ramosissimus* induced more severe and uniform lesions (hard, consolidated lesions) in the lungs of inoculated goats than did *T viride*, which induced large, softer consolidated lesions and smaller lesions in 3 goats. *Trichoderma viride* appeared to be more variable in its pathogenicity from goat to goat; however, that fungus appeared to be more invasive within the thoracic lymphatic system on the basis of thoracic lymph node isolation data. This variability between *M ramosissimus* and *T viride* isolation from the lymph nodes may be in part a consequence of the smaller spore size of *T viride*, or other unknown immunologic factors. There may also be different pathogenic routes by which these 2 fungal agents induce lesions. Among the goat groups in the present study, there appeared to be no difference in induced antibody synthesis. It is interesting that the goats inoculated with spores and exposed to dust had significantly more fungal spores per gram of consolidated lung lesion than goats that were inoculated with spores and not exposed to dust. This phenomenon occurred with both genera of fungi used. Lung macrophages may engulf and become filled with fine dust, thereby making them less effective at engulfing and inactivating the spores of either fungus. We have investigated this phenomenon before; we have examined the effects of aerosolized coarse dust particles (size range, 0.89 µm to 355.6 µm; mean, 100.03 µm) on lung clearance of *Pasteurella multocida* and *Mannheimia haemolytica* in goats.[31] The objective of that study was to determine whether the inhalation of large quantities of feedyard dust predisposed goats to pulmonary bacterial proliferation; the results indicated that the pulmonary clearance of 2 potential bacterial pathogens in dust-exposed goats was not significantly different from that in goats that were not exposed to dust.[31] However, pulmonary clearance of fine particulates is a great deal slower than clearance of coarse particles.[32] Also, in rat lung macrophages, it has been shown that there is a lengthened inflammatory response with enhanced chemotactic substance release and production of proinflammatory cytokines associated with fine particle inhalation.[33] These phenomena could then allow fungal spores to avoid phagocytosis. In the present study, the mean ± SD particle diameter of the dust inhaled by the goats was < 7.72 ± 0.69 µm, and the spore-treated goats had significantly more spores per gram of consolidated lung lesion tissue than did the spore-treated goats that were not exposed to dust.

a. Ivomec, MSD AGVET, Merck & Co Inc, Rahway, NJ.
b. Sulfaquinoxaline (20%) sodium solution, Loveland Industries, Greeley, Colo.
c. ACD solution (dextrose hydrous, 2.45 g; sodium citrate hydrous, 2.20 g; citric acid anhydrous, 0.73 g; distilled water to 100 mL), Western Medical Supply Co, Arcadia, Calif.
d. Alsever's solution (dextrose, 20.50 g; sodium citrate dihydrate, 8 g; citric acid monohydrate, 0.55 g; sodium chloride, 4.20 g; and distilled water to 1 L; autoclaved for 15 minutes at 121°C, at 6.9 kPa), American Standard, Steris Corp, Erie, Pa.
e. Falcon plastic Petri plates, Becton, Dickinson & Co, Franklin Lakes, NJ.
f. Bright line hemacytometer, Hausser Scientific, Horsham, Pa.
g. Falcon plastic multiwell plate, Becton, Dickinson & Co, Franklin Lakes, NJ.
h. Pasteur pipette, VWR, West Chester, Pa.
i. FCS 16000-044, GIBCO, Grand Island, NY.
j. Leitz Optometric Inc, Richards, Tex.
k. Falcon plastic snap-cap (5-mL) test tube, Becton, Dickinson & Co, Franklin Lakes, NJ.
l. Ultraspec II, LKB Biochrom Ltd, Cambridge, UK.
m. Agarose Sea Kem, FMC BioProducts, Rockland, Me.
n. Auto-Gel cutter, Grafar Corp, Detroit, Mich.
o. braun-Sonic, model 1510, B. Braun Instruments, San Francisco, Calif.
p. Microscope light, Bausch & Lomb, Rochester, NY.
q. Phosphate-buffered saline solution without glucose, Sigma Chemical Co, St Louis, Mo.
r. Sterile cotton-tipped plastic applicators, Puritan 25-806-2PC, Hardwood Products Co, Guilford, Mass.
s. Centrifuge RC-5B, Sorvall, Wilmington, Del.
t. Carbonate buffer solution, Sigma Chemical Co, St Louis, Mo.
u. Maxi-Sorb Nunc immunomodules, Nalge Nunc International, Rochester, NY.
v. Falcon microtiter plates, Becton, Dickinson & Co, Franklin Lakes, NJ.
w. Tween-20, Sigma Chemical Co, St Louis, Mo.
x. SuperBlock, Pierce, Rockford, Ill.
y. Horseradish peroxidase–conjugated rabbit anti-goat IgG (H+L), Southern Biotech, Birmingham, Ala.
z. 1-Step Slow TMB-ELISA, Pierce, Rockford, Ill.
aa. Sulfuric acid, Sigma Chemical Co, St Louis, Mo.
bb. MRX plate reader, Dynex Technologies Inc, Chantilly, Va.
cc. Micro Grinding Systems Inc, Little Rock, Ark.
dd. Mastersizer 2000 particle size analyzer, Malvern Instruments Inc, Southborough, Me.
ee. Autoclave, Amsco 2021 Eagle series, Steris Corp, Healthcare Division, Erie, Pa.
ff. Custom-fabricated canvas tent, Wolfe Canvas, Amarillo, Tex.
gg. Jet-O-Mizer, Fluid Energy Processing & Equipment Co, Hatfield, Pa.

hh. Five-stage cyclone device, In-Tox Products, Albuquerque, NM.
ii. Vaccum pump, Andersen Sampler Inc, Atlanta, Ga.
jj. Field isolates (*Mucor ramosissimus* and *Trichoderma viride*) identified by Center for Indoor Air Research, Health Sciences Center, Texas Tech University, Lubbock, Tex.
kk. Malt extract agar, Difco Laboratories, Detroit, Mich.
ll. Atropine sulfate LA (15 mg/mL), Neogen Corp, Lexington, Ky.
mm. Butorphanol tartrate (10 mg/mL), Fort Dodge Animal Health, Fort Dodge, Iowa.
nn. Ketamine HC (100 mg/mL), Fort Dodge Animal Health, Fort Dodge, Iowa.
oo. Custom-fabricated speculum made of polyvinylchloride pipe (2.7 X 20 cm) fitted with a clip pen light, Agricultural Research Laboratory, USDA, Bushland, Tex.
pp. Falcon plastic 0.1-mL pipettes, Becton, Dickinson & Co, Franklin Lakes, NJ.
qq. Rectal thermometer, hi-speed digital rechargeable, GLA, San Luis Obispo, Calif.
rr. EDTA, Sigma Chemical Co, St Louis, Mo.
ss. Unopette dilution chambers No. 365856 for WBC determination by manual methods, Becton, Dickinson & Co, Franklin Lakes, NJ.
tt. Beuthanasia-D Special, Schering-Plough Animal Health, Union, NJ.
uu. Custom-fabricated walk-in environmental incubator, Agricultural Research Laboratory, USDA, Bushland, Tex.
vv. Sartorius top-loading balance 1409 MP8, Brinkmann Instruments Co, Westbury, NY.
ww. Tenbroeck all-glass grinder, Kimble/Kontes, Vineland, NJ.
xx. Formaldehyde (37 wt% solution in water), ACS Reagent, Aldrich, Milwaukee, Wis.
yy. H&E stain, Sigma Chemical Co, St Louis, Mo.
zz. Gomori methenamine silver stain, Sigma Chemical Co, St Louis, Mo.

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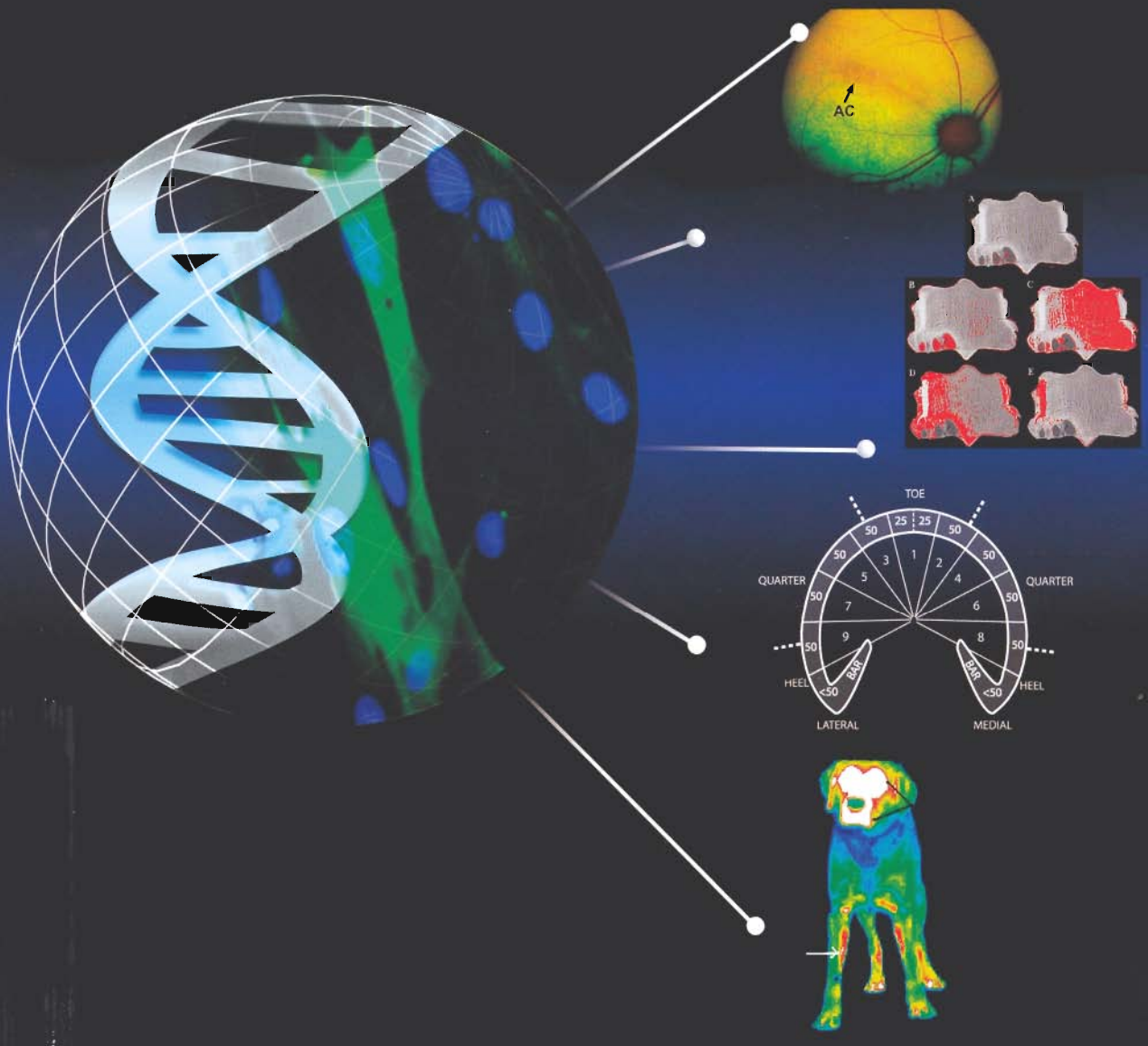

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